Immigration delays but does not prevent adaptation following environmental change: experimental evidence

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Abstract

An important and pressing goal in conservation is to determine how to effectively manage populations experiencing environmental change. When populations begin to decline, extinction will occur unless populations can adapt in response to natural selection, a process called evolutionary rescue. Theory predicts that immigration can delay extinction and provide novel genetic material that may reduce inbreeding depression and facilitate adaptation. However, when potential source populations have not experienced the new environment before (*i.e.,* are naive), immigration can counteract selection and constrain adaptation. This study evaluated the effects of immigration of naive individuals on evolutionary rescue using the red flour beetle, *Tribolium castaneum,* as a model system. Small populations were exposed to a challenging environment, and three immigration rates (0, 1, or 5 migrants per generation) were implemented with migrants from a benign environment. Following an initial decline in population size across all treatments, populations receiving no immigration gained a positive growth rate one generation earlier than those with immigration, illustrating the constraining effects of immigration on adaptation. After seven generations, a reciprocal transplant experiment found evidence for local adaptation regardless of immigration rate. Thus, while the immigration of naive individuals briefly delayed adaptation, it did not increase extinction risk or prevent adaptation to severe and abrupt environmental change.

Keywords: evolutionary rescue, dispersal, *Tribolium castaneum*, gene flow, translocation

1 | Introduction

A critical goal of conservation biology is to establish how to effectively manage populations in human-altered habitats. Environmental change such as pollution (Loria *et al.*, 2019), deforestation (Foster *et al.*, 2021), and fragmentation (Cheptou *et al.*, 2017) can reduce habitat quality and connectivity, which can decrease the average fitness of residing populations. If fitness drops below the replacement rate, populations will decline in size and will approach extinction if the downward trend persists. Immigration, either through maintenance of dispersal corridors or translocations, is one approach readily available to conservation practitioners to support declining populations, particularly in fragmented landscapes (Frankham *et al.*, 2019).

The conservation literature recommends an immigration rate of one to ten migrants per generation for isolated populations (Mills & Allendorf, 1996). More specifically, one migrant per generation aims to offset population divergence that occurs through drift, while five migrants per generation aims to prevent inbreeding depression and maintain adaptive potential in changing environments (Frankham *et al.*, 2019). Ideally, immigration can occur consistently through dispersal corridors, and if translocations are needed, consistent immigration should be sustained. However, these recommendations have been tested only partially. Laboratory experiments have demonstrated the benefits of immigration when comparing isolated populations to populations that received a single immigration event under scenarios of inbreeding (Spielman & Frankham, 1992) or low diversity populations facing environmental change (Hufbauer *et al.*, 2015). Only one study we know of, Newman & Tallmon (2001), tested immigration that occurred every generation. They assessed rates of 1 or 2.5 migrants per

generation in newly-isolated mustard plant populations, and found that the rate of 1 migrant/gen was sufficient to maintain genetic variation and decrease inbreeding when compared to isolated populations (Newman & Tallmon, 2001). In this era of global change, it is important to also test these recommendations in more severe environmental conditions.

A separate but related body of literature focuses on how populations declining due to exposure to challenging environments can avoid extinction through adaptive evolution, a process called evolutionary rescue (Gomulkiewicz & Holt, 1995; Carlson *et al.*, 2014). Examples include adaptation in weed or insect populations to pesticides, antibiotic resistance in bacteria, or invertebrate adaptation to metal pollution in streams (Bell, 2017). Experiments show that immigration or admixture can enhance the probability of evolutionary rescue if populations are exposed to a challenging environment (Hufbauer *et al.*, 2015; Durkee *et al.*, 2023). However, gene flow from maladapted immigrants is well known to limit adaptation, slowing the increase in frequency of adaptive alleles and reducing fitness (*i.e.,* outbreeding depression) (Slatkin, 1987; Lenormand, 2002; Brady *et al.*, 2019).

To reduce the likelihood of outbreeding depression, habitat matching between source and recipient populations is recommended (Edelaar & Bolnick, 2012; Frankham *et al.*, 2019). However, this is often not possible, particularly under scenarios of novel environmental change (Aitken & Whitlock, 2013) or when the species of concern is rare (Frankham *et al.*, 2019). Even when migrants are not adapted to their new habitat, for example when individuals from a geographically distant population (*e.g.,* Hedrick & Fredrickson, 2010) or from captivity (*e.g.,* Crone et al., 2007) are used, every-generation immigration of 1 to 5 individuals is meant to be low enough to maintain genetic diversity and adaptive potential of populations (Newman &

Tallmon, 2001; Frankham *et al.*, 2019). However, to our knowledge, no study has tested the recommended immigration rates of one vs. five migrants per generation experimentally in the context of severe environmental change.

Here, we evaluated how immigration recommendations from the conservation literature (Frankham *et al.*, 2019) impacted evolutionary rescue (Carlson *et al.*, 2014) in a challenging environment. To do this, we used red flour beetle (*Tribolium castaneum*) populations without a history of inbreeding or bottlenecks to model a situation where large, diverse populations experience sudden habitat change, causing them to become both small and fragmented. In habitats like these that have limited space and degraded resources, population recovery may be constrained by density-dependent processes like competition for food and space (Osmond & de Mazancourt, 2013; Nordstrom *et al.*, 2023). We exposed these populations to a new, challenging environment, and then implemented three different rates of immigration: 0, 1, or 5 individuals each generation from a large population not adapted to the new environment. We evaluated both density-dependent and density-independent population growth to tease apart the impacts of negative density dependence and adaptation. Our experiment was designed to address the following question: In populations experiencing severe environmental change, how do different immigration rates affect ***(i)*** population size and growth rate over time, ***(ii)*** the timing of evolutionary rescue, and ***(iii)*** adaptation and the associated consequences of adapting to the challenging environment?

100 ## 2 | Methods

101 To evaluate the question posed in the introduction, we carried out an experiment where
102 we subjected replicate populations to a challenging environment that caused populations to
103 decline in size, and therefore would have led to extinction unless populations adapted
104 sufficiently to the challenging conditions. Hereafter, we refer to this as the rescue experiment.
105 We created a genetically diverse *T. castaneum* stock population comprised of lineages
106 described in Durkee et al. (2023). The stock population had been reared on standard media
107 (19:1 wheat flour to brewer's yeast) and in standard environmental conditions (31°C and 50-
108 80% humidity) in 4x4x6cm patches for nine non-overlapping generations prior to the start of
109 the rescue experiment. Each generation was five weeks long, with adult oviposition occurring
110 on day 35. To start the experiment, we initiated 25 independent populations from the stock
111 population. These independent populations were used to initiate experimental populations and
112 maintain populations to serve as a source of migrants (hereafter, source populations).
113
114 *Rescue experiment initiation and propagation*
115 **Experimental populations** were each initiated with 50 adults that were placed onto a
116 single patch of challenging environment created using media containing the pyrethroid
117 insecticide deltamethrin (DeltaDust, Bayer). These two events together represent a sudden
118 environmental change causing both habitat degradation and population fragmentation
119 simultaneously. The experimental patches contained 2.968ppm of deltamethrin, a
120 concentration that a dose-response curve created from preliminary experiments indicated
121 would decrease population growth from a typical value of 2, or doubling every generation, to

122 0.40 (Figure S1). This growth rate guaranteed population extinction in 10 generations without
123 adaptation or immigration based on simulations with the stochastic Ricker model developed for
124 *T. castaneum* by Melbourne & Hastings (2008). Adults were 36 days old at population initiation,
125 and they were given 24 hours to mate and oviposit in the experimental patches. Adults were
126 discarded after oviposition, and offspring were then given 35 days to develop. Experimental
127 populations were propagated for 7 generations in this way, with populations censused
128 completely each generation on day 35. In total, 75 experimental populations were initiated
129 across three temporal blocks.

130 **Source populations** of migrants were initiated with 150 adults from the stock
131 population and then maintained at 150 adults each subsequent generation, regardless of the
132 number of offspring produced. This approximates a population with a carrying capacity of 150
133 individuals. Source populations were each maintained on a single patch containing standard dry
134 media, a benign environment for *T. castaneum*. Thus, these individuals were not exposed to the
135 challenging environment present in the experimental patches. Hereafter, we refer to these
136 individuals as naive migrants, to refer to both evolutionary and behavioral naivety. We
137 expected that the source populations would have a growth rate greater than the replacement
138 rate. However, when this did not occur, populations were supplemented with individuals from
139 the stock population to maintain the oviposition density at 150 adults per patch. This
140 supplementation occurred at the start of the oviposition period (day 35), after immigration to
141 the experimental populations occurred. After the first generation, two source populations were
142 initialized with 110 individuals due to limited availability of stock individuals. In total, 25 source

populations were maintained total across the three temporal blocks, one for each group of immigration treatments.

Three immigration rates were used in the rescue experiment: zero, one, or five migrants every generation. Each generation, experimental populations received migrants from the source population on day 35 before the oviposition period. Immigration occurred by moving individuals using a paintbrush from the source population to each experimental patch. Across the 7 experimental generations, 6 migrants were added to the 1-migrant populations and 30 migrants were added to the 5-migrant populations in total. Each immigration treatment was replicated 25 times, in 25 separate groups comprised of three treatments each, split across three temporal blocks. Within each group, treatments were set up sequentially using the same pool of adult beetles, and propagated for the first four generations in the following order: 1-migrant, 5-migrants, and 0-migrants. As this was not random, we tested if this order mattered in a follow-up experiment and found no significant effect of patch order on the growth rate the following generation (Figure S2).

The two immigration recommendations of one or five migrants per generation are based on either natural dispersal or translocation. Thus, in order to approximate natural dispersal and make our results applicable to either case, we used migrants with higher demonstrated dispersal ability to represent individuals that would be more likely to leave the source patch and move to a new patch in the absence of translocation (Edelaar & Bolnick, 2012; Cote *et al.*, 2022). **Dispersal arrays** were created to select dispersing individuals. On day 34 during generations 1-6, habitat patches housing source populations were connected to 3-patch dispersal arrays connected with 2mm holes, creating a 4-patch dispersal array. Hereafter, we

165 refer to the original source patch as patch 1 and the subsequent patches, in order, as patches 2-
166 4, which contained media that had been previously used to rear beetles for 35 days to keep
167 resource availability consistent throughout the array. Adult beetles were given 24hrs to
168 disperse out of patch 1. On day 35, all adults were censused in each of the four patches of the
169 dispersal array (see Figure S3). Individuals that dispersed to patches 2-4 were used as migrants
170 (hereafter called dispersing migrants) with preference given to individuals that dispersed to the
171 furthest patch (*i.e.,* individuals were first chosen from patch 4, then patch 3, then 2).
172
173 *Reciprocal transplant experiment*
174 After seven generations, we terminated the rescue experiment, and a reciprocal
175 transplant experiment was completed using the 58 extant experimental populations and the 25
176 source populations to assess adaptation to the challenging environment and potential costs to
177 adaptation in the source environment. We measured the growth rate of each population for
178 one generation in each of three environments: standard media (0ppm, hereafter "source
179 environment"), media containing the concentration of deltamethrin present in the challenging
180 environment of the rescue experiment (2.968ppm, hereafter "experimental environment"), and
181 media containing a higher concentration of deltamethrin (5.641ppm, hereafter "severe
182 environment"). Using the dose-response curve created in preliminary experiments (Figure S1),
183 this severe concentration was intended to reduce the growth rate of naive populations to zero.
184 To standardize maternal and environmental effects, all extant populations were placed
185 at a constant density of 40 individuals on standard media for one generation during generation
186 8 (hereafter, "common garden" generation). For three populations, a density of fewer than 40

individuals was used due to small population size ($N < 50$) at the conclusion of the rescue experiment. All adults were kept within their experimental populations, *i.e.,* no mixing occurred within or across treatments.

The reciprocal transplant experiment occurred in generation 9 using the adult offspring from the common garden generation, and replication depended on the number of available individuals (see Figure S6). The growth rate of each experimental population was measured in each of the three test environments (source, experimental, and severe) with an initial population size of 40. In total, there were 23-24 replicates of populations from the 0-migrant populations (average replication of 1.1/population) across the three environments, 19-20 replicates (average replication of 1.2/population) from the 1-migrant populations, 22 replicates for the 5-migrant treatment (average replication of 1.1/population), and 24-25 replicates for the source populations (replication of 1/population). Additional replication occurred when enough individuals (*i.e.,* 160 or more) were available (Figure S6).

Data analysis

All statistical analyses were performed in R version 4.1.2 (R Core Team, 2021). Population size through time was used to assess population dynamics throughout the rescue experiment. Populations were considered rescued if they grew to a population size greater than 50, and maintained a growth rate of greater than 1 (the replacement rate) for at least two of the final four generations. This allowed for decreases in population size due to negative density dependence. Extinction risk and survival times were quantified using a survival analysis with the *survival* package (Therneau 2021). Extinct populations were defined as those with zero adults at

209 census during generations 1-6 or one adult by generation 7. Overall extinction rates were
210 calculated as raw percentages with confidence intervals around each percentage for each
211 treatment.

212 Population growth was calculated as the mean number of surviving adult offspring per
213 individual in the population, *i.e.,* census population size measured on day 35 (N_t) divided by the
214 initial population size (N_{t-1}), or $\frac{N_t}{N_{t-1}}$. We evaluated both density-dependent and density-
215 independent growth rates through time using a linear mixed model with the *lme4* package
216 (Bates *et al.*, 2015). We included immigration treatment (0 migrants, 1 migrant, or 5 migrants
217 per generation), time (t) in generations (from 1 to 7 generations, categorical), and initial
218 population size (N_{t-1}, continuous) as fixed effects, and temporal block (block 1, block 2, block
219 3), source population (nested within block), and population identity (nested within source
220 population) as random effects. Growth rate was square root transformed to accommodate zero
221 values and to ensure that the equal variance assumption was satisfied.

222 From this model, we generated estimates of both density-dependent and density-
223 independent population growth rates. To estimate density-dependent growth rates, we set the
224 values for the initial population size (an explanatory variable in the model) to vary for each
225 generation and treatment combination, matching the observed mean initial population sizes.
226 These estimates were used to describe the population growth data and quantify the effects of
227 immigration treatment in a finite habitat by considering the constraints of competition for
228 resources and cannibalism that affected the populations in our experiment (Pointer *et al.*,
229 2021). To estimate density-independent growth rates from the fitted model, we set the values
230 of initial population size to zero (*i.e.*, $N_{t-1} = 0$) for every generation and treatment, which is

equivalent to estimating the y-intercepts (see Figure S5). These density-independent estimates allowed us to track adaptation to the environment by revealing the change in intrinsic fitness of individuals in the population unfettered by the effects of density. We expected the density-independent growth rates to be higher than the density-dependent estimates due to the known negative density dependence in *T. castaneum* (Melbourne & Hastings 2008). Confidence intervals for both density-dependent and density-independent growth rate estimates were estimated by parametric bootstrap (percentile method) using the *bootMer* function with 5000 simulations in the *lme4* package (Bates *et al.*, 2015). Comparisons between treatments were made by estimating confidence intervals by parametric bootstrap (percentile method) for the difference in means between treatments within each generation using the *simpleboot* and *boot* packages with 5000 simulations (Peng, 2019; Canty & Ripley, 2022).

The timing of evolutionary rescue was assessed in each treatment group using a Generalized Additive Mixed (GAM) model (Wood, 2017) similar to Olazcuaga *et al.* (2023). Only rescued populations were considered in this analysis. We modeled the population size of rescued populations using a negative binomial distribution to account for overdispersion using the package *mgcv* (Wood, 2004, 2011). To allow for the relationship between the population size and time to be nonlinear, we modeled population size as a smooth function of generation with the number of knots set to 4, which was chosen to maximize the R-squared value. Explanatory variables also included immigration treatment and the interaction of treatment and generation as fixed effects, allowing for a smooth function for each treatment. The full model included population identity and temporal blocks as random effects. The best-fit model

was chosen by comparing to reduced models using the *compareML* function from the *itsadug* package (van Rij *et al.*, 2022).

We used the reciprocal transplant data to further evaluate adaptation to the experimental environment, as well as potential fitness costs of adaptation in a benign environment. Growth rate in the reciprocal transplant experiment was used as the response variable in a linear mixed effects model that included population type (source, 0-migrant, 1-migrant and 5-migrant), test environment (source, experimental, severe), and the interaction between population type and test environment as fixed effects. Population identity was included as a random effect nested within temporal block. Comparisons within each environment and among immigration treatments were made using 95% bootstrapped confidence intervals (percentile method) for the difference in means, which were generated from 5000 iterations.

3 | Results

Population size and extinction

During the rescue experiment, a majority of populations experienced evolutionary rescue (Figure 1, black lines), and extinction probability was similar for all three treatments (Figure 1, red lines). The difference in the extinction risk was statistically unclear across treatments throughout the experiment (Figure S4). Additionally, there was no clear difference in survival time among treatments ($\chi^2_2 = 0.1$, p = 1.00).

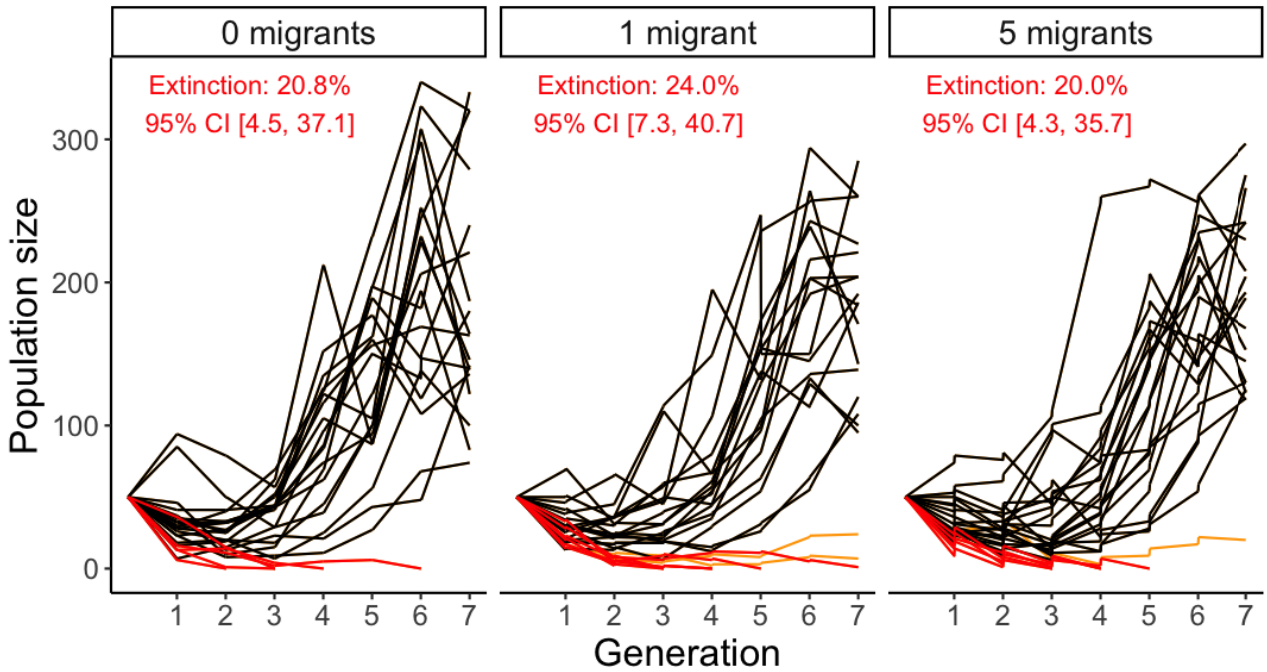
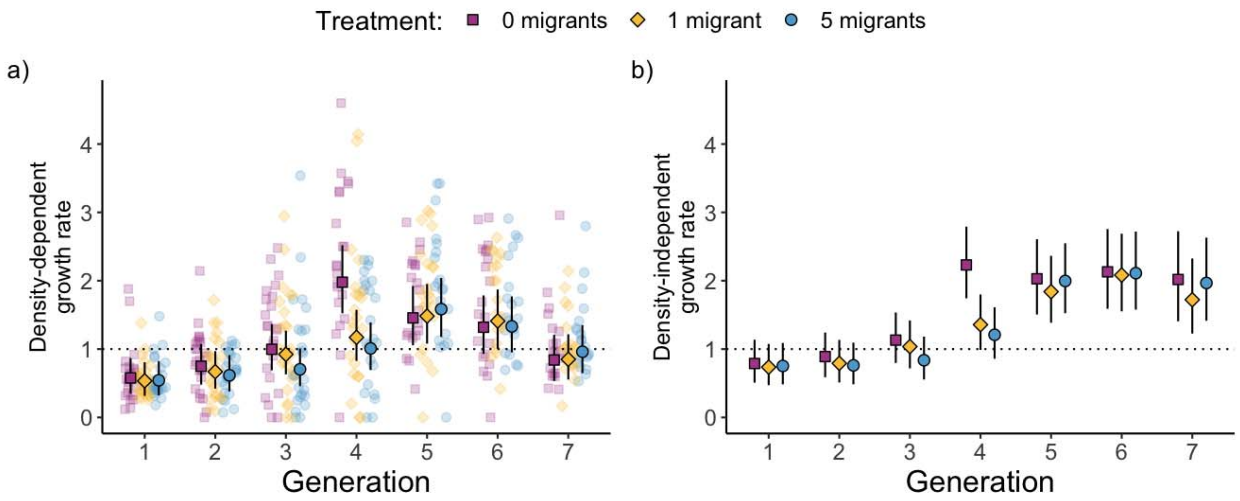


Figure 1. Population size through time in discrete generations for the 0-migrant, 1-migrant, and 5-migrant per generation treatments during the 7-generation rescue experiment. Each black line represents a rescued population, each orange line represents an extant population that was not considered rescued, and each red line represents a population that went extinct or had a population size of one individual in generation 7. Immigration events are indicated with instantaneous increases in population size, which occurred at the start of generations 1-6. Extinction rates (% of red lines) and simple confidence intervals around each percent are provided.

3.2 | Growth rate through time

Density-dependent growth rates increased through generation four, then decreased again as population density increased (Figure 2a, Table S1). Density-independent growth rates

288 were below or close to the replacement rate (growth rate of 1) in generations 1-3 (Figure 2b;
289 confidence intervals overlap with dashed line). This changed in generation 4, when the 0-
290 migrant treatment had a mean density-independent growth rate much larger than the
291 replacement rate, illustrating rapid adaptation within those populations (Figure 2). In
292 generation 4, density-independent growth rate in the 0-migrant populations was on average
293 0.808 (95% CI [0.207, 1.453]) higher than the 1-migrant populations and 0.969 (0.478, 1.573)
294 higher than the 5-migrant populations. By generation 5, all treatments gained and then
295 maintained a mean density-independent growth rate greater than 1, showing adaptation to the
296 environment. The comparison between the density-independent and density-dependent
297 estimates of growth rates suggest that decrease in the latter by generation 7 was likely due to
298 the effects of negative density dependence, which increased as populations grew (see Figures 1
299 & 3).
300



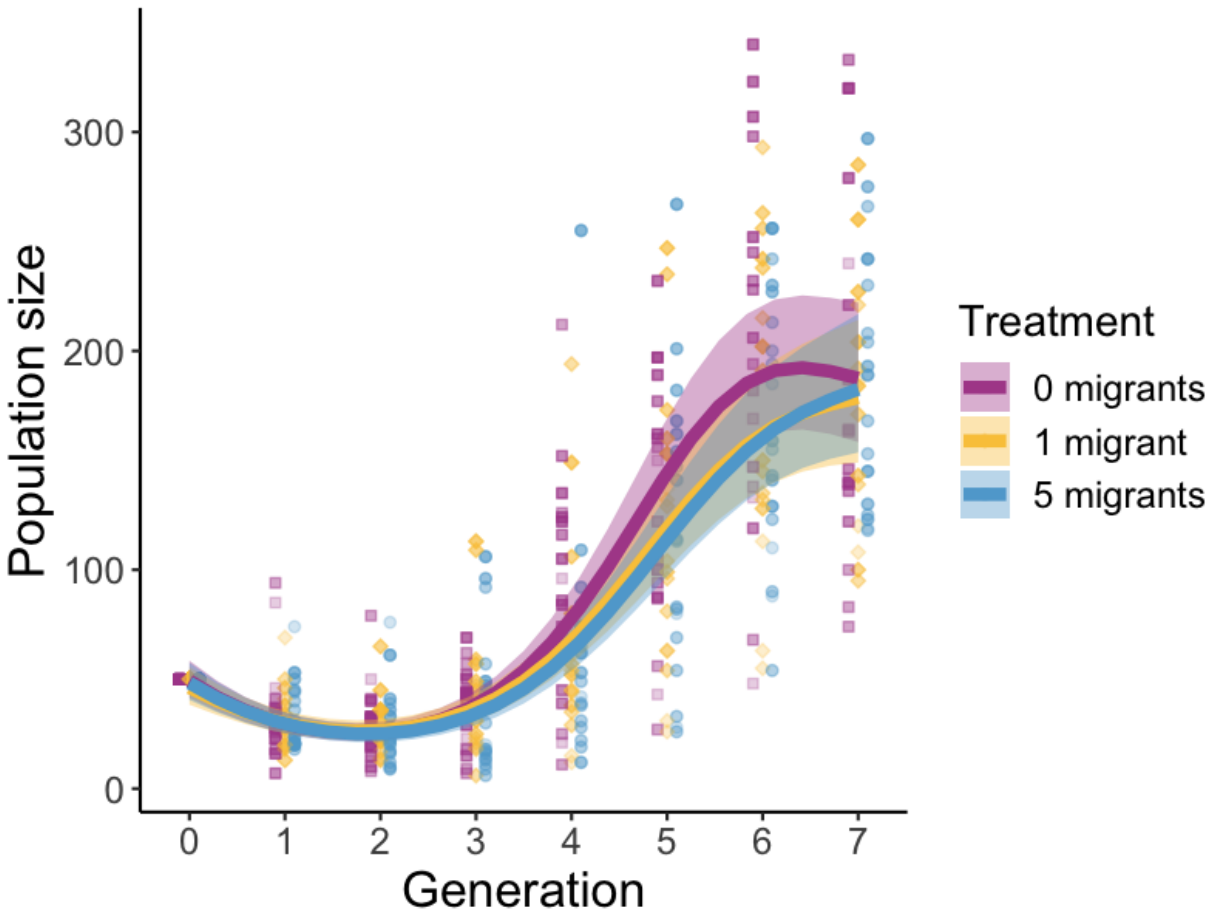
301

302 **Figure 2. Density-dependent growth rate (a) and density-independent growth rate (b) throughout the**
303 **rescue experiment (generation 1 to 7).** Each point outlined in black is the predicted mean for each

treatment (purple square = 0 migrants, yellow diamond = 1 migrant, and blue circle = 5 migrants). Error bars are bootstrapped 95% confidence intervals. Points without black outlines are raw growth rate values, and each point represents one population. The dotted line represents a growth rate of 1, the replacement rate. Two estimates for growth rate are presented: **a)** the density-dependent estimates are predictions from the linear mixed model using average initial population size for each generation and treatment combination, used to incorporate the effects of negative density-dependence and provide estimates that describe the raw data (shown), and **b)** the density-independent estimates (*i.e.,* using an initial population size of 0), showing adaptation over time.

Timing of evolutionary rescue

Among the rescued populations only (black lines in Figure 1), the analysis of population size with a GAM model revealed a shallow U-shaped curve (Figure 3). The estimated curves from the best-fit model, which included treatment and the interaction of treatment and generation as fixed effects and population identity as a random effect, are shown in Figure 4. The interaction between immigration treatment and generation was significant ($\chi^2_3 > 330$, $p < 0.0001$), with the 0-migrant populations growing slightly faster than populations receiving migrants (Figure 3). The 1-migrant and 5-migrant treatments maintained similar population trajectories throughout the experiment.



323

324 **Figure 3. Evolutioanry rescue analysis.** Population size of rescued populations receiving no

325 migrants (purple line, squares), one migrant (yellow line, diamonds), or five migrants (blue line,

326 circles) through time in generations estimated by the best-fit GAM model. Each point

327 represents one population, which are staggered to reduce overlap. The lines are estimates of

328 mean population size for the three immigration treatments from the best-fit GAM model. The

329 shaded region around each line is the 95% confidence interval given by the GAM model.

330

331 *Adaptation and associated costs*

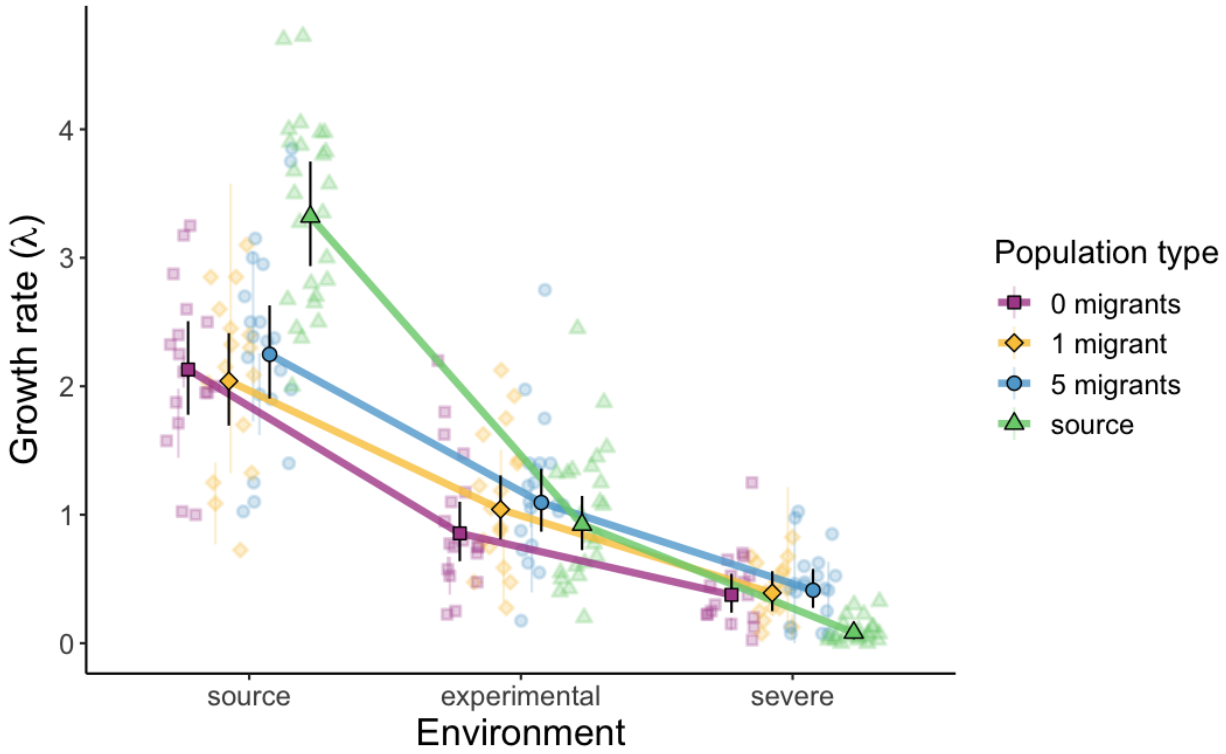
332 Growth rate in the reciprocal transplant experiment was influenced by an interaction

333 between environment and population type ($F_{6, 168.04} = 25.16$, $p < 0.0001$), in a pattern showing

that adaptation to the challenging environment resulted in reduced fitness in the benign source environment (Figure 4). There, the experimental populations had lower growth rates on average compared to the source populations (95% $CI_{0\text{-migrant} - \text{source}}$ [-0.855, -1.628]; 95% $CI_{1\text{-migrant} - \text{source}}$ [-0.911, -1.748]; 95% $CI_{5\text{-migrant} - \text{source}}$ [-0.641, -1.482]), and a higher growth rate in the severe habitat (95% $CI_{0\text{-migrant} - \text{source}}$ [0.442, 0.171]; 95% $CI_{1\text{-migrant} - \text{source}}$ [0.424, 0.210]; 95% $CI_{5\text{-migrant} - \text{source}}$ [0.464, 0.216]). Additionally, the 5-migrant populations performed marginally better than the 0-migrant populations in the experimental environment (95% $CI_{0\text{-migrant} - 5\text{-migrant}}$ [-0.0675, 0.572]).

Unexpectedly, the experimental and source populations performed similarly in the challenging experimental environment (Figure 4, all confidence intervals of the differences overlap with zero: 95% $CI_{0\text{-migrant} - \text{source}}$ [-0.404, 0.209]; 95% $CI_{1\text{-migrant} - \text{source}}$ [-0.220, 0.403]; 95% $CI_{5\text{-migrant} - \text{source}}$ [-0.153, 0.452]). The experimental populations did not perform as well as expected based on their growth rates at the end of the rescue experiment (Figure 2). Our expectation for growth rates were based on predictions with initial population size of 40 (the size used in the reciprocal transplant) from the linear mixed model created for the growth rate data in the rescue experiment. Those predicted values were 1.73, 95% CI [1.19, 2.34], 1.46 [1.02, 1.98], and 1.68 [1.21, 2.23] for the 0-migrant, 1-migrant, and 5-migrant treatments, respectively. The average growth rates we observed in the reciprocal transplant experiment in the experimental environment were lower than these estimates, with the means 0.86 [0.64, 1.10], 1.04 [0.81, 1.31], 1.09 [0.87, 1.36] that fall at the edge or outside the predicted values and CIs, and confidence intervals that overlap only slightly.

355 Furthermore, source populations did not perform as poorly as expected. We expected
356 them to perform similarly to the first generation of the experimental populations in the rescue
357 experiment prior to adaptation. The prediction given N_{t-1} = 40 was 0.62 [0.38, 0.92] for the 0-
358 migrant populations, while the mean growth rate of source populations was 0.92 [0.72, 1.15].
359 The CIs overlap partially, but the overlap does not include the mean, confirming that the source
360 growth rates were unexpectedly high.



361

362 **Figure 4. Reciprocal transplant experiment results.** Growth rate was measured in three
363 environments: the source environment (standard dry media), the challenging experimental
364 environment (2.968ppm deltamethrin, the concentration used during the rescue experiment),
365 and a severe environment containing a higher concentration (5.641ppm). Each point outlined in
366 black is the model-estimated mean for each treatment (purple square = no migrants, yellow
367 diamond = 1 migrant, blue circle = 5 migrants, and green triangle = source population). Black

error bars are bootstrapped 95% confidence intervals; for the source populations in the severe environment (green), the black error bars are not visible because they are too small to be seen. Thick lines connecting these points emphasize the treatment-environment interaction. Each point without the black outline represents a single population, with the light error bars representing raw 95% confidence intervals across replicates; points without error bars have no replicates. Points are staggered and jittered to reduce overlap.

4 | Discussion

Our study evaluated the effects of different immigration rates on adaptation to a new, challenging environment using experimental populations of *T. castaneum*. We evaluated differences between populations receiving two immigration rates (1 or 5 migrants per generation) with isolated populations that received no immigration. The migants were not adapted to the challenging environment, allowing us to examine the degree to which immigration can limit adaptation (Savolainen *et al.*, 2013). While the experimental populations declined in size initially, leading some populations to go extinct, around 80% of populations were able to persist for the duration of the 7-generation experiment. A majority of these populations had growth rates above the replacement rate and large population sizes, characteristic of evolutionary rescue. We also found that extinction rate was similar regardless of immigration. However, we observed that growth rates increased one generation sooner in the isolated populations compared to those with immigration, indicating that outcrossing with migrants that were not adapted to the challenging environment likely delayed adaptation. This delay was brief, as the following generation, growth rates were comparable across the different

390 immigration treatments. We also tested for adaptation using a reciprocal transplant
391 experiment, where we observed a pattern of local adaptation, with experimental populations
392 having higher growth rates compared to naive populations in a severe environment, and lower
393 growth rates than the naive populations in a benign environment, which suggests that
394 adaptation to the challenging environment was associated with a cost.
395
396 *Observed delay in adaptation*
397 Populations without immigration gained a density-independent growth rate greater
398 than the replacement rate one generation before the populations with immigration. The
399 following generation, all populations grew at a similar rate regardless of immigration. Taken
400 together, we can conclude immigration of individuals not adapted to a new, challenging
401 environment, even at a reasonably high level, did not prevent adaptation, only that it slowed
402 the process. Such delayed adaptation could be detrimental to populations experiencing urgent,
403 time-sensitive extinction risk due to small size (Melbourne & Hastings, 2008). However, for
404 populations not at immediate risk of extinction, such a delay may be inconsequential, and
405 worth the potential increase in genetic diversity that occurs through immigration, which may
406 improve the ability of the population to adapt to future environmental changes. This finding of
407 only a brief (one generation) delay of adaptation has practical implications. Naive migrants are
408 often more feasible to obtain, either from captive rearing programs (Crone *et al.,* 2007) or from
409 large source populations living in a different habitat (*e.g.,* different spider mite host plants in
410 Bisschop et al., 2019) or geographic region (*e.g.,* geographically isolated guppy populations in
411 Fitzpatrick et al., 2020), when habitat matching is not possible.

412 Our findings are consistent with refuge strategies to delay evolution of pest resistance
413 to toxins, such as in crops genetically modified to express *Bacillus thuringiensis* (*Bt*). The
414 immigration of susceptible individuals from sections of fields planted in non-*Bt* crops, or
415 growing crops in areas with plentiful alternative host plants for the pest has been proven to be
416 highly effective (Carrière *et al.*, 2012). In those situations, particularly with 50% or more of a
417 pest population being susceptible, resistance can be successfully managed for multiple
418 generations (Tabashnik *et al.*, 2008). Given our much smaller proportion of susceptible
419 individuals, it makes sense that the delay of the evolution of resistance was brief.
420

421 *Reciprocal transplant experiment: thoughts on adaptation and genetic load*

422 We observed consistent signals of adaptation in the experimental populations in the
423 second half of the rescue experiment and in the severe environment of the reciprocal
424 transplant experiment. However, we did not expect the experimental and source populations
425 to have similar growth rates in the experimental environment – the experimental populations
426 performed worse than expected, and the source populations performed better than expected,
427 as detailed in the results. We did observe that the populations receiving five migrants per
428 generation performed marginally better than the isolated populations. This suggests that there
429 may be a benefit of immigration in the longer term by preventing or alleviating genetic load
430 (Hedrick & Garcia-Dorado, 2016; Stewart *et al.*, 2017).

431 However, the performance of the experimental populations overall was lower than
432 expected, and this is challenging to explain. It does not seem likely to be a general effect of
433 genetic load, as just two generations prior, in generation 7 of the rescue experiment,

434 populations demonstrated density-independent growth rates well above the replacement rate.
435 The relatively high growth rate of the source populations in the challenging environment may
436 be related to adaptation to a different environmental challenge, high density, which affected
437 source populations. The source populations were maintained at a consistently higher density (*N*
438 > 150 on average for all 7 generations; Figure S3) than the experimental populations (*N* > 150 in
439 generations 4-7 only; Figure 1) in habitat patches of the same size. High density creates a
440 stressful environment for *T. castaneum,* which results in adults releasing stress hormone and
441 engaging in increased cannibalism (Hirashima *et al.*, 1993; Pointer *et al.*, 2021). This heightened
442 competition for twice as many generations may have resulted in adaptation to higher density,
443 perhaps through evolution of increased cannibalism (Stevens, 1989) along with the use of
444 cannibalism to persist in the challenging, high-density environment (Via, 1999). In our reciprocal
445 transplant experiment, increased cannibalism may have enabled the source populations to
446 perform comparably to the experimental populations in the challenging experimental
447 environment as well.
448
449 *Reciprocal transplant experiment: costs to adaptation*
450 The adaptation of the experimental populations to the challenging environment, while
451 clear when examining the density-independent growth rates, was only apparent in the
452 reciprocal transplant experiment in the severe environment. Adaptation to moderate-to-high
453 concentrations of deltamethrin is well-documented in pest populations of *T. castaneum*
454 (Collins, 1998; Stuart *et al.*, 1998; Kalsi & Palli, 2015), and in our study, adaptation enabled
455 tolerance of higher concentration of the insecticide. The low performance of insecticide-

456 adapted populations in a benign environment suggests a cost to adaptation, which has been
457 documented in the literature for other insect species. For example, detoxification of pyrethroid
458 insecticides through the over-expression of cytochrome P450 enzymes leads to reduced
459 fecundity and fertility in mosquitoes (Tchouakui *et al.*, 2020). While a direct cost to pyrethroid
460 resistance in *T. castaneum* has to our knowledge not been documented, it seems likely that
461 there would be a fitness cost given that the cytochrome P450 pathway is also involved in
462 resistance to deltamethrin in the species (Kalsi & Palli, 2015).
463
464 *Considerations for future experiments*
465 The bottleneck events that occurred during the rescue experiment, some as small as 10
466 individuals, will likely have longer-term consequences (Frankham *et al.*, 1999; Olazcuaga *et al.*,
467 2023). Census population size is almost always larger than the effective population size (the
468 median N_e/N_c ratio across plant and animal taxa is approximated to be 0.1; for *T. castaneum,* it
469 is estimated to be between 0.5-0.9) and bottlenecks of $N_e < 50$ can result in reductions in
470 genetic variation and increases in inbreeding (Wade, 1980; Jamieson & Allendorf, 2012). For
471 this reason, we suggest that future work examine the effects of demographic bottlenecks of
472 this severity by propagating experiments like this for longer (*i.e.,* more than 8 generations).
473 Longer-term experiments would be able to test the hypothesis that the benefits of immigration
474 become more apparent (*i.e.,* genetic load may be lower in populations with immigration as
475 opposed to those without) over longer time scales. Such experiments would help shed light on
476 the longer-term impacts of immigration for small and isolated populations, an important
477 question when considering whether to implement translocations in declining natural

478 populations (Frankham, 2015, 2016). Genomic methods would also provide important insights
479 for monitoring the genetic contributions of migrants and their effects on population fitness
480 (Whiteley *et al.*, 2015).
481
482 *Conclusions*
483 Overall, this study explored the effects of immigration of naive individuals on adaptation
484 of previously large and outbred populations following sudden environmental degradation and
485 habitat fragmentation. Of course, the ideal solution would be to restore the habitat, and this
486 study provides support for using immigration as a tool to sustain populations for multiple
487 generations until restoration can occur (Whiteley *et al.*, 2015; Frankham *et al.*, 2019). We found
488 that though immigration slowed adaptation in the short-term, extant populations with and
489 without immigration were on average adapted to the new and challenging environment after
490 seven generations. Broadly, the balance between migration and selection can determine the
491 magnitude of adaptation in natural populations as well as the amount of genetic variation
492 present among connected populations (Kawecki & Ebert, 2004; Yeaman & Jarvis, 2006;
493 Savolainen *et al.*, 2013; Reid *et al.*, 2021). Thus, we demonstrate that evolutionary rescue can
494 occur both with and without consistent immigration from a naive source population. Our
495 findings suggest the deleterious effects of naive migrants are short-lived, and should not
496 prevent managers from implementing consistent immigration through corridors or
497 translocations to maintain genetic diversity and increase adaptive potential in populations
498 exposed to novel environmental change.
499

Acknowledgements

This work was supported by the U.S. National Science Foundation Graduate Research Fellowship No. 006784 to LFD and Grant Nos. 1930222 and 1930650 to BAM and RAH, respectively. We thank Sarah DeLacey, Freddie Habarecht, Mayra Contreras-Hernandez, Desiree Moore, and Rachel Szymanski for assistance maintaining laboratory *T. castaneum* colonies used in this experiment. We thank XX anonymous reviewers for their constructive feedback.

Conflict of Interest

The authors declare no conflicts of interest.

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